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14. ABSTRACT The overall objective of this Discovery Award is to explore the hypothesis the ketogenic diet regulates neuronal excitability by influencing potassium channel activity via the auxiliary potassium channel subunit Kvβ2. To test this hypothesis we have examining the impact of the ketogenic diet on mice in which the gene that encodes Kvβ2 has been deleted (Kvβ2 KO mice) using an in vitro model of seizure induction. We have examined the first cohort of Kvβ2 KO mice and somewhat surprisingly it appears that Kvβ2 KO mice on a normal diet (ND) exhibit a lower frequency of in vitro bursting activity (seizures) which is reversed by treatment with the ketogenic diet (KD). Conversely, the latency to the first in vitro burst event is reduced in Kvβ2 KO mice on ND compared to wild-type mice on a ND. This effect was also reversed by the ketogenic diet. We are continuing to increase the sample size for this cohort and are developing additional computational tools to further analyze the seizure data.					
15. SUBJECT TERMS Epilepsy, Ketogenic Diet, Seizure Disorder, Potassium Channels, Neurophysiology.					
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1. Introduction:

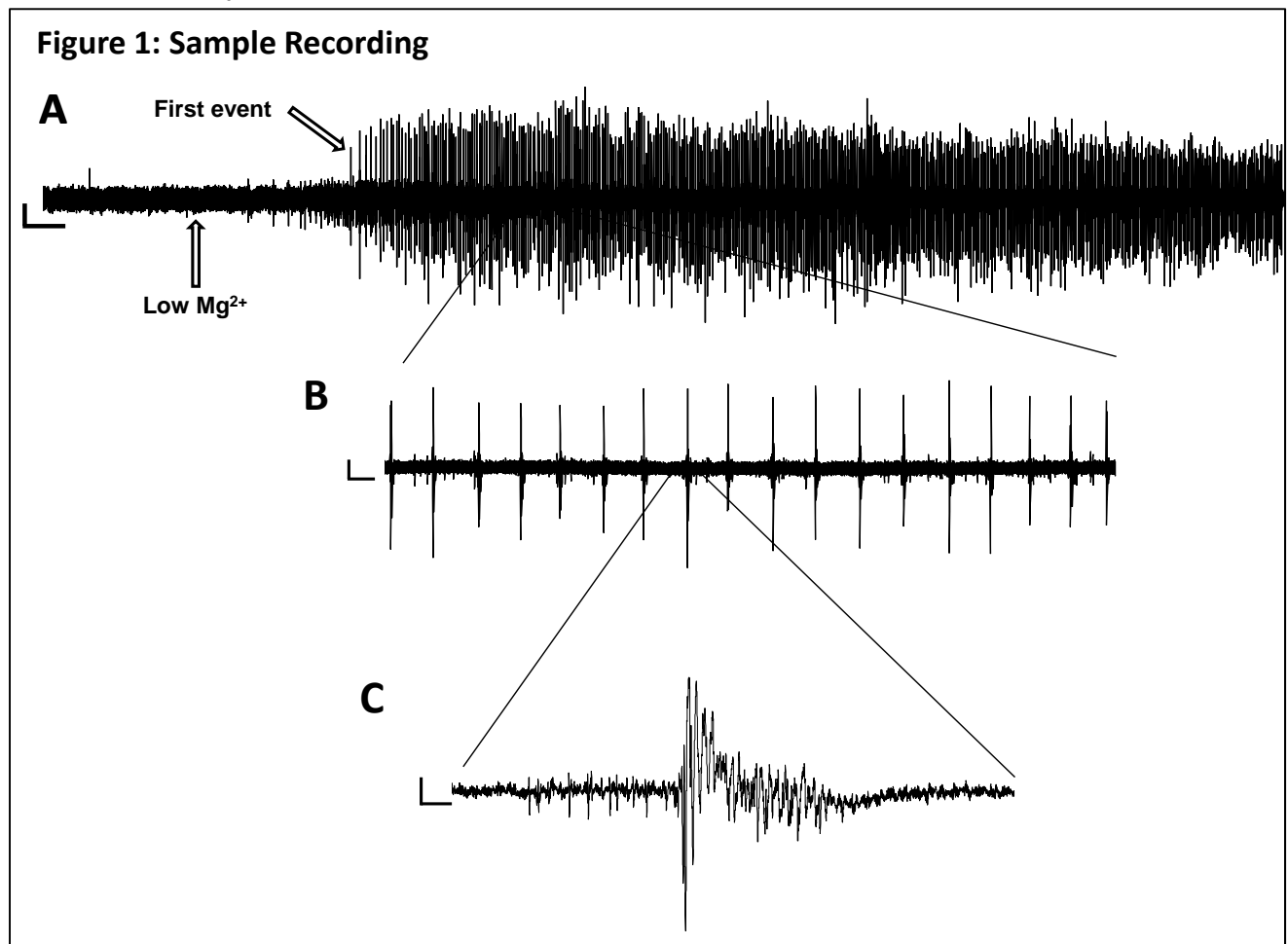
The overall goal of this Discovery Award is to explore the hypothesis that the ketogenic diet (KD), which is used to treat epilepsy (primarily in children) exerts a positive effect on seizure activity by regulating neuronal excitability via a subclass of potassium auxiliary subunits known as Kv β subunits. Therefore we have been treating genetically engineered mice that lack these subunits (specifically the Kv β 2 subunit) with the KD and examining the interaction of diet and seizure activity in vitro.

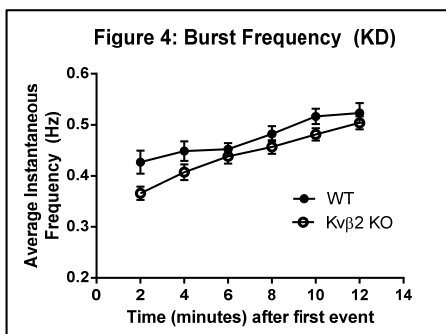
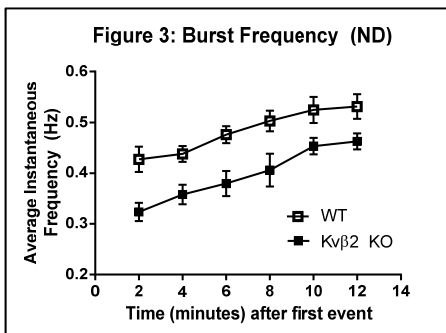
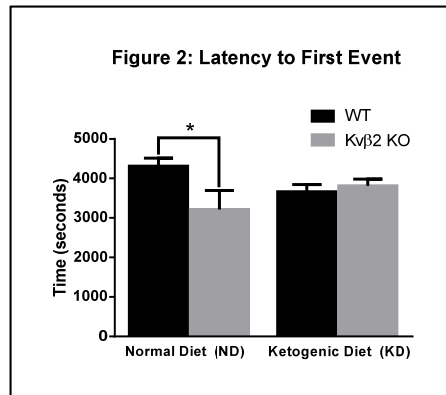
2. Keywords:

Epilepsy, Ketogenic Diet, Seizure Disorder, Potassium Channels, Neurophysiology

3. Overall Project summary:

Within the last year we have established a reasonably sized breeding colony of mice to support our research goals. As anticipated we have not encountered any issues in maintaining/genotyping this colony. To date we have made a significant number of recordings from mice lacking the Kv β 2 subunit (Kv β 2 KO mice) to compare to wild-type (WT) littermate controls. A sample recording from a typical experiment is presented in Figure 1 below (scale bars: 100 μ V and 5 min, 30 sec, 10 ms for A, B & C respectively).





Recordings are made in CA3 region of the hippocampal slices prepared under standard conditions (e.g. (Moore, Throesch et al. 2011)). After a stable baseline was recording, the normal artificial cerebrospinal fluid (aCSF) was replaced with aCSF which contained 0.5 mM magnesium (Low Mg^{2+} in Fig 1A above) and was continuously perfused throughout the remainder of the recording. Typically, after several minutes of low Mg^{2+} spontaneous rhythmic burst events were observed (Fig 1B) which consisted of a biphasic waveform containing multiple spikes of varying amplitude (Fig 1C). For these experiments

mice (Kvβ2 KO & WT) were fed either a normal diet (Purina 5010) or a KD diet that consisted of ~ 75% fat (BioServ F3666) for a period of up to six weeks. Using the latency to the first burst event as an indicator of seizure susceptibility we found that on the normal diet the Kvβ2 KO mice appear to be more susceptible under normal diet (ND) conditions when compared to WT littermates (Figure 2). However there was no significant difference between KO mice and WT mice on the ketogenic diet (Figure 2). In addition we measured the rate at which the bursting occurred during the recording. Figures 3 and 4 present the average instantaneous frequency of bursts recorded for 10 minutes after the occurrence of the first event (2 min bins). Somewhat surprisingly, the Kvβ2 KO mice exhibit a significant lower frequency of events when maintained on ND when compared to WT mice (Figure 3). This difference in burst frequency was eliminated when the Kvβ2 KO mice were maintained on a KD (Figure 4).

4. Key Research Accomplishments:

Nothing to report

5. Conclusion:

Although our study is still ongoing and therefore definitive conclusions will need to be deferred, at this point we make the interim conclusion that in our in vitro seizure model, deletion of Kvβ2 appears to modestly reduce seizure susceptibility while also reducing the rate of repetitive bursting. Paradoxically, under ketogenic conditions hippocampal slices from the Kvβ2 KO mice appear to be similar to WT mice in terms of latency to the first event and event frequency. At present our sample size for this study is reasonable (min 7 slices per group), over the next six months we will increase this number to ensure that these findings are in fact reproducible. In addition, we are developing computational tools to analyze the complex waveforms generated during the bursting. At present we are investigating to what extent if any the amplitude and inter-burst frequency of the events are modulated by loss of Kvβ2. Finally we are conducting similar experiments using the Kvβ2^{ΔY90F} mice.

6. Publications, Abstracts and Presentations:

Nothing to report

7. Inventions, Patents and Licenses:

Nothing to report

8. Reportable outcomes:

Nothing to report

9. Other Achievements:

Nothing to report

10. References:

Moore, S. J., et al. (2011). "Of mice and intrinsic excitability: genetic background affects the size of the postburst afterhyperpolarization in CA1 pyramidal neurons." Journal of Neurophysiology **106**(3): 1570-1580.

11. Appendices:

None